The effect of *Chlamydia* on translocated *Chlamydia*-naïve koalas: a case study

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ABSTRACT

Thirty *Chlamydia*-free koalas, *Phascolarctos cinereus,* were moved from French Island National Park to three forests near Ballarat (Victoria). Chlamydial exposure and infection were monitored by antibody Enzyme-linked Immunosorbent Assay (ELISA), Direct Immunofluorescence (DIF) and Polymerase Chain Reaction (PCR) of swabs; its impact evaluated by clinical examination.

Chlamydia was not detected on French Island. At the end of the study, 16 out of 17 koalas were *Chlamydia* antibody positive, and 11 out of 16 were also positive for the presence of *Chlamydia* in the uro-genital tract. *C. pecorum* infected seven out of nine koalas, one out of nine were infected by *C. pecorum* and *C. pneumoniae* and one out of nine by *C. pneumoniae* alone.

This translocation trial shows a high incidence of infection of the translocated koalas, suggesting that the movement of *Chlamydia*-free animals to areas where the status of the disease is unknown, or the movement of infected animals to other sites where koalas are present, should not be considered as a management option without detailed pre-release research.

Further studies should focus on ascertaining the longer term impact of the disease on individuals and population dynamic of this species.

Key words *Chlamydia* infection, animal relocation, marsupials

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Introduction

Translocation

Translocation is defined as the 'human-mediated movement of living organisms from one area, with release in another' (IUCN/SSC 2013); translocations of many wildlife species are used for a diverse array of reasons, and recently, translocation of species has been proposed as a method to mitigate the effect of climate change as it causes range shift through ecosystems (Considine 2011; Gallagher *et al.* 2015; Minteer and Collins 2010).

Moreover, as anthropogenic environmental and landscape changes (deforestation, urbanisation, fires, climate change) have caused many species to overpopulate with negative consequences to their habitat and other species (Garrott *et al.* 1993; Menkhorst 2008), translocation is used to manage the individuals from high to less densely populated sites (Australian and New Zealand Environment and Conservation Council (ANZECC) 1998). This is the case for *Odocoileus* sp. (Black-tailed Deer) (O'Bryan and McCullough 1985), *Loxodonta* sp. (Elephant) (Pinter-Wollman *et al.* 2009) as well as koalas, *Phascolarctos cinereus* (Backhouse and Crouch 1990; Lee *et al.* 1990; Menkhorst *et al.* 1998).

Translocations from overpopulated sites, where koalas over-browse preferred fodder tree species, have occurred since the 1920s (Martin and Handasyde 1999) and is still used occasionally. Thousands of koalas were moved to more than 200 island and mainland release sites, in the states of Victoria and South Australia (Menkhorst *et al.* 1998; Short 2009), as a response by the Department of Environment to reduce over-browsing in other areas (Australian and New Zealand Environment and Conservation Council (ANZECC) 1998; Backhouse and Crouch 1990; Menkhorst *et al.* 1998). However, more recently, the policy of the current Victorian government to overpopulation and over-browsing in the The Otways is translocation, fertility control and euthanasia of those animals in declining health (Department of Environment Land Water and Planning 2015).

Contrary to Victoria, koalas were declared vulnerable in Queensland and New South Wales and Australian Capital Territory in 2012 (Department of Environment and Energy 2016) as populations are declining due to anthropogenic activities detrimentally impacting koala habitat, climate change, urbanisation, dogs, cars and disease (McAlpine *et al.* 2015). Translocation of koalas is the main management tool used to deal with koalas when land development for housing or roads takes place in koala habitat (Council of the City of Gold Coast 2015; Department of Environment and Heritage Protection 2014b).

The World Conservation Union/Species Survival Commission (IUCN/SSC) developed the IUCN Guidelines for the re-introduction and translocation of living organisms in 1998 (IUCN/SSC) and more recently in 2013 (IUCN/SSC). These aim at ensuring that proper actions and research are undertaken before and after any translocation. One of the risks associated with translocation is the transmission of diseases, which can occur from translocated animals to the resident population and *vice versa* (Boyce *et al.* 2011; Dein *et al.* 1995; Fraser *et al.* 2009; Short *et al.* 1992; Woodford and Rossiter 1994). Moreover, increased prevalence of disease in translocated animals has been linked to stress (Dickens *et al.* 2009; Fraser *et al.* 2009).

Chlamydia in koalas

Species affecting koalas are *C. pneumoniae* and *C. pecorum* (Everett *et al.* 1999; Glassick *et al.* 1996). The former mainly causes respiratory tract infections, but it can also be detected in both uro-genital and ocular sites (Wardrop *et al.* 1999). The latter is the main agent responsible for chronic urogenital infection (also known as wet bottom or dirty tail), also responsible for bladder, kidneys and reproductive tract infections; often resulting in infertility and death (Everett *et al.* 1999; Govendir *et al.* 2012). In addition, there is some reported evidence of *C. pecorum* being responsible for a higher infection prevalence than *C. pneumoniae* (Jackson *et al.* 1999; Koala Research Network (KRN) 2011; Kollipara *et al.* 2013). However, Jackson *et al.* (1999) examined two free-ranging koala populations in Queensland; koalas tested at Mutdapilly showed higher *C. pecorum* infection levels, while at Coombabah both, C. pecorum and *C. pneumoniae*, were detected at equal rates. Some studies have also shown the higher infection grade of *C. pecorum* compare to *C. pneumoniae,* in most infected koalas (Blanshard *et al.* 2008; Polkinghorne *et al.* 2013).

Presence of *Chlamydia* was detected in the past in Victorian koalas on the mainland (Lavin *et al.* 1990; Martin and Cross 1997; Menkhorst *et al.* 1998; Obendorf 1983; Timms *et al.* 1996), however, there is little information about the current prevalence. In a recent study carried out on 288 koalas (Patterson *et al.* 2015), *C. pecorum* was detected in 41% and 25% of animals sampled in the populations of Raymond Island and Mt Eccles National Park, respectively; *C. pneumoniae* was not detected at either site. Moreover, as determined in this and previous studies (Emmins 1996; Koala Research Network (KRN) 2011; Martin and Handasyde 1999; McColl *et al.* 1984), *Chlamydia* was absent on French Island. Patterson *et al.* (2015) also found that wet bottom and urogenital ultrasound-detected abnormalities were linked to *C. pecorum* positive koalas; nevertheless, urogenital infection and ultrasound abnormalities were also found in *C. pecorum* negative animals and were not detected in all *Chlamydia* positive koalas.

Outbreaks of overt signs of chlamydial disease in koalas have been attributed to stress (Amis 2014; Canfield *et al.* 1991; Department of Environment and Heritage Protection 2014a; Melzer *et al.* 2000; Weigler *et al.* 1988), and this link has also been documented in humans, as well as other animal species (Barton and Iwama 1991; Cohen and Williamson 1991; Koolhaas *et al.* 1999; Lafferty and Holt 2003; McCallum and Dobson 2002; Thomason *et al.* 2013). Phillips (2000) argues that *Chlamydia* can limit overpopulation of koalas in undisturbed habitats, however it can have a devastating effect where anthropogenic changes have occurred (Rhodes *et al.* 2011). Links between environmental modifications, stress and diseases expression are unclear, therefore, there is a need to investigate if a correlation exists (Brearley *et al.* 2013).

The focus of the work presented here is on the impact of *Chlamydia* on 30 *Chlamydia*-naïve koalas This was part of a two year project which investigated the outcome of a translocation of koalas from French Island National Park to three state forests near Ballarat (Victoria) as part of the then Department of Environment. Due to the previous Victorian Government's historical limited monitoring of the ongoing translocation programs from French Island to mainland Victoria, which occurred over decades, the focus of this study was to ascertain the effect of this management tool on the health of the relocated animals.

Materials and methods

Koalas

Twenty female (ten sub-adult and ten adult) and ten male (five sub-adult and five adult) koalas were caught on French Island in collaboration with personnel of the former Department of Natural Resources and Environment (DNRE) Victoria as part of the past ongoing translocation program to alleviate over-browsing of *E. viminalis*. Sub-adult koalas, in this study, were animals between one and three years of age, established by tooth wear (Gordon 1991). Five of the mature females had back-young and one had a pouch-young at the time of capture and release. Juveniles were released with the adults but were not included in the study. Only the adults caught on the island were radio-collared as part of this study (Figure 1)

The initial of the codename given to the released koalas corresponded to the first initial of the name of the forest into which they were released (e.g. Elisabeth was released into ESF). For the progeny conceived and born from translocated mothers at the release sites, the mother's name plus the suffix "by" for "baby" (e.g. Elisabethby was Elisabeth's young), was used.

Health examinations

Three health examinations were carried out during the study period. The first was undertaken on French Island on the day of capture; the second and third at six and nineteen months post-release (April 1998, May 1999). These examinations were carried out post mating seasons.

After capture on French Island, koalas were weighed, head length was measured (from the back of the occipital crest to the tip of the nose) and tooth wear was used to

estimate age. Health examination included assessing any external signs of disease or injury and body condition as defined by (Martin 1985). A blood sample was taken for the detection of chlamydial antibodies using Enzyme-linked Immunosorbent Assay (ELISA).

During the second and third examination, health status was assessed as above and a second blood sample was taken. Also, uro-genital swabs were taken for detection of chlamydial antigen using Direct Immunofluorescence (DIF) and cell culture. Nine koalas were also swabbed for detection of *Chlamydia* antigens (using ELISA), and DNA by Polymerase Chain Reaction (PCR).

During all examinations, koalas were neither anaesthetised nor sedated. They were kept inside a canvas bag and only the genital area was exposed for swab collection. This method appeared satisfactory in managing animals' stress. After examination, koalas were immediately released at the base of the same tree where they were caught and observed to ensure that they were safely climbing the tree.

In December 1998 four back-young of released females were caught and fitted with radio-collars, and blood samples for chlamydial antibody detection were taken. In May 1999 these tests were repeated.

Chlamydia antibody detection ELISA

A 2 ml blood sample was taken from the cephalic vein (Figure 2) and sent chilled to Dr John Emmins at the Monash Medical School. The presence of antibodies was detected using the genus-specific *Chlamydia* Enzyme Linked Immunosorbent Assay (ELISA), (Emmins 1996; Emmins and Turner 1992). This test assumes titre value of 0= negative, 1= background reactor, 2= low reactor, >2 positive. In the present study a titre of 2 or above was considered 'positive' (+ve) because all koalas were negative when released at the translocation sites.

Chlamydial antigen detection

For each koala, two 15 cm disposable aluminium shaft-buffered swabs were inserted into the everted penis and in the uro-genital sinus of females and rotated several times. One of the swabs was smeared onto a slide, whilst the second was placed into a tube containing *Chlamydia* transport medium. Samples were transported on dry ice and then subjected to Direct Immunofluorescence (DIF), or cell culture if results with DIF were negative. The methods used for the testing are described in (Martin 1998). All the samples collected were adequate for testing.

Polymerase Chain Reaction (PCR)

A disposable aluminium shaft-buffered swab was used to swab the genital area of nine koalas previously tested to be DIF or cell culture positive. Three koalas were chosen among those released into CFS, two koalas from LLSF and four from ESF. *Chlamydia* species was determined

Figure 1. One of the radio-collared koalas that was part of the study. (Photograph by F. Santamaria)

Figure 2. Flavia Santamaria and Dr John Emmins collecting a blood sample for ELISA test, from the cephalic vein of one of the koalas. (Photograph by R. Schlagloth)

by Polymerase Chain Reaction (PCR). Test for presence of any *Chlamydia* using a genus -specific 16SrRNA PCR assay; positives were speciated using a nested *C. pecorum*-specific or *C. pneumoniae*-specific ompA PCR assay. This test was carried out at Queensland University of Technology by Professor Timms. All the samples collected were adequate for testing.

Sites

Koalas (n=30) were caught from areas, across French Island National Park, dominated by *Eucalyptus viminalis* and were released into three mixed eucalypt forests around Ballarat (mainland Victoria) (Figure 3). These forests (Figure 4) were Enfield State Forest (ESF) (n=10: 6 females and 4 males), Creswick State Forest (CSF) (n=10: 5 females and 5 males) and Lal Lal State Forest (LLSF) (n=10; 8 females and 2 males. The resident koalas were not tested for *Chlamydia*,

Results

First examination (French Island)

All 30 koalas examined on French Island, prior to translocation, were in good condition as indicated by a physical examination, and were *Chlamydia* negative (ELISA < 1).

Second examination (six months post-translocation)

In April 1998 (second examination), chlamydial antibodies (ELISA>1) were detected in 56% (9/16) of females and 33% (3/9) of males. Seroprevalence varied between the three forests (Figure 5); 20% (2/10) of koalas in ESF showed ELISA units greater than 1, contrasting noticeably with the percentages of infection at CSF 60% (6/10) and LLSF 80% (4/5). A Kruskal-Wallis test showed that there was a significant difference in chlamydial antibody titre among koalas in the three forests (p= 0.036 n=25). However, DIF and/or cell culture, (n=25), were negative for all koalas. It appeared that all of the koalas were healthy and in good condition; the weight of all but two was either maintained or increased.

Pouch-young were observed in some koalas at ESF (3/6), at CSF (n=1/6). All koalas appeared to be healthy.

Third examination (19 months post-translocation)

Fourteen koalas were blood tested and 13 were swabbed in May 1999. *Chlamydia* antibody titre (ELISA) increased for all but one koala in the three forests, 11/12 females showed an increase between 2 and 8 ELISA units. Both males showed a similar increase with values between 3 and 8 ELISA units. One female (Elisabeth) in ESF did not show any increase (ELISA units = 0) (Figure 6).

A Wilcoxon test performed on the two *Chlamydia* titre results for the 2nd and 3rd examination showed a highly significant difference in the titre levels across the two examinations (p=0.001 n=14). DIF and/or cell culture for detection of chlamydial organisms at the uro-genital site were positive n 9/13 (69%) koalas. The weight of all but two koalas was maintained, only that of two females in ESF increased during the 13 months after the first examination.

Figure 3. Koalas were released into mixed eucalypt forests. (Photograph by R. Schlagloth)

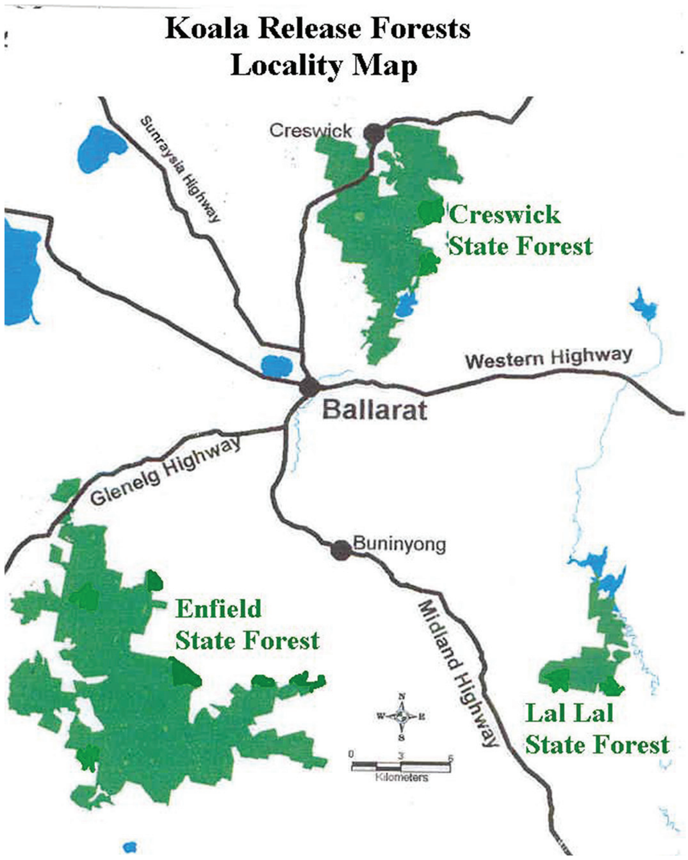

Figure 4. Map showing the location of the three State Forests, in the Ballarat region, where koalas were released.

Progeny of translocated animals

Between August and October 1998, six females were seen with female back-young at ESF (3/6), at CSF (2/6) and at LLSF (1/3). In December 1998 four (two from ESF, one

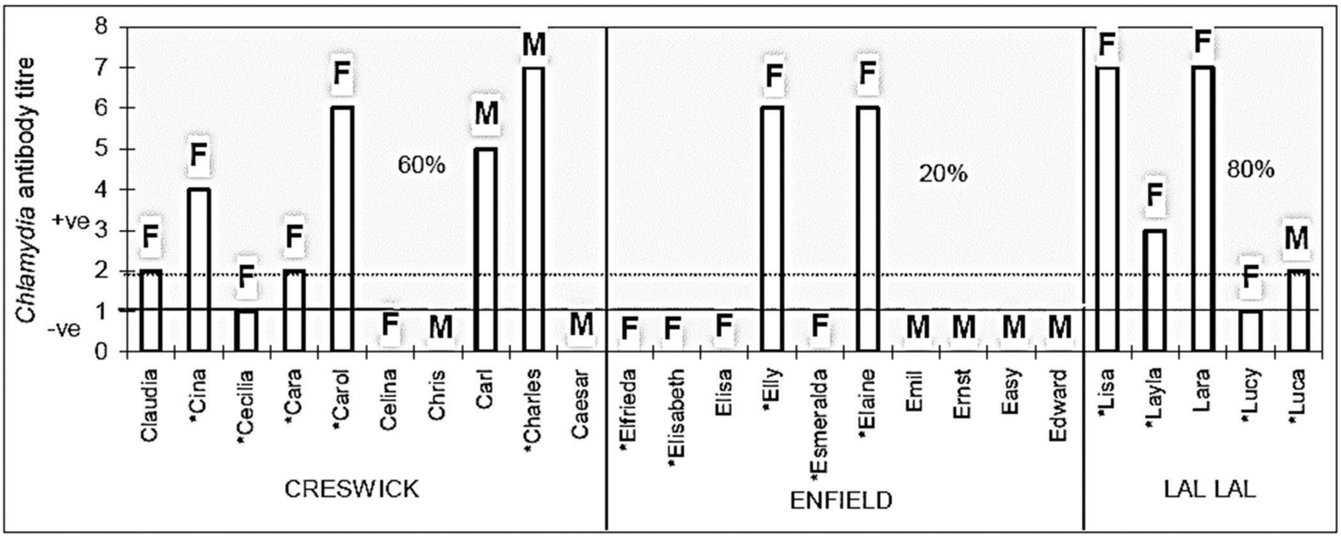

Figure 5. *Chlamydia* antibody response (ELISA) and prevalence in 25 of the 30 male (M) and female (F) koalas translocated from French Island to mainland Victoria, six months post-translocation. The character * Indicates koalas followed to the end of the study. The line ______ indicates the minimal vector reactor below which *Chlamydia* antibody titre is negative in this research. The line - - - - - indicates the minimal vector reactor below which *Chlamydia* antibody titre is negative according to ELISA test guidelines.

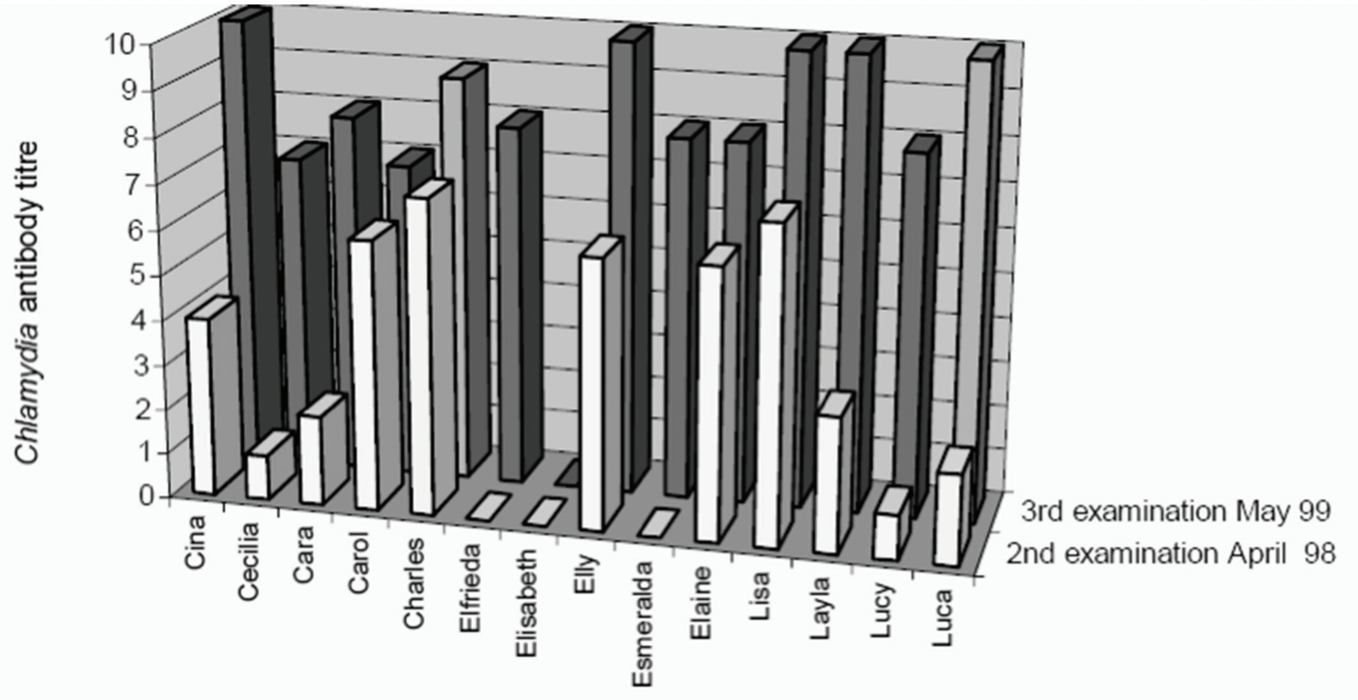

Figure 6. Comparison of *Chlamydia* antibody response (ELISA) of 14 koalas after six (2nd examination) and 19 months (3rd examination) post-translocation. M = males

from CSF, one from LLSF) were fitted with radio-collars and blood samples were taken. Juveniles' antibody titre was comparable to that of their mothers (Table 1).

Before the third examination (May 1999) one juvenile (from ESF) lost its collar, and therefore no blood test was carried out. Of the three remaining juveniles (from CSF, ESF and LLSF) one was positive in December 1998 (ELISA units=3) but was negative in May 1999 (ELISA units=0) indicating that this may have been a false positive result (Dr Emmins personal communication). This was in contrast with her mother, whose chlamydial antibody titre was very high (ELISA units=10) at the last examination (May 1999). ELISA titre of the other juvenile, which was negative (ELISA units=0) in December 1998 test, was high in the May 1999 test (ELISA units=7). DIF for this koala showed presence of chlamydial antigen on urogenital swab. A third juvenile whose ELISA titre was high in December (ELISA units=7), still showed the same high titre value in May 1999. Cell culture for this koala was also positive.

Table 1. Chlamydia antibody titres (measured by ELISA) for back-young and their mothers (December 1998).

Back-young	Chlamydia antibody titre		Mother
Esmeraldaby	0	0	Esmeralda
Elisabethby	0	0	Elisabeth
Lisaby	7	7	Lisa
Cinaby	3	4	Cina

Chlamydia test results in the three forests

Table 2 shows results from the second and last ELISA and DIF and/or cell culture tests for all koalas, including juveniles. Fewer koalas were tested due to loss or death of animals between second and last examination. The last examination showed that proportion of chlamydial-positive koalas was comparable among forests. Nevertheless, there was a sharp increase in the number of animals with increased ELISA titre between the second and last examinations for the three forests.

The presence of chlamydial organism in the uro-genital site was also detected in 11 out of 16 koalas tested.

Chlamydia species

PCR was performed for nine koalas that were positive for either DIF or cell culture from ESF (adult female n=4), from CSF (adult female n=2; adult male n=1) and from LLSF (adult female n=1; female back-young n=1). Eight females (including back-young) were infected with *C. pecorum*; one of these (mother of the back-young) had a double infection (*C. pecorum* and *C. pneumoniae*). One dead pouch young was found in one of these *C. pecorum*-positive female's pouch (Elly). The male was infected with *C. pneumoniae* only (Table 3). The breeding success rate decreased between the first (6/16) and the last (live n=1 and dead=1 out of 12) breeding seasons. However, no overt signs of disease were visible during the whole study.

Discussion

Results of this study confirmed the absence of chlamydial antibodies in all koalas tested on French Island prior to translocation to the mainland. This is consistent with previous reports of the island's *Chlamydia*-free status (Emmins 1996; Koala Research Network (KRN) 2011; Martin and Handasyde 1999; McColl *et al.* 1984; Patterson *et al.* 2015),

Presence of antibodies after the first mating season (6 months post-release) in 56% of individuals, in the three forests, suggested that the resident koalas in the selected forests were *Chlamydia*-positive. However, compared with the other two forests, lower infection rates occurred in ESF. This might be explained by either absence of interaction between released and resident koalas, due to low animal density in the area (extensive fire in 1995 burned 107 km^2 of forest), or due to the mating between translocated koalas, or a lower prevalence of infected resident males.

Difference in infection rate amongst sexes may be explained by the pressure exerted by resident male koalas. This pressure can influence the ability of the 'immigrant' animals to mate (Gordon *et al.* 1990). Eventually, mating with infected resident animals spread the infection through the released colony as previously shown by (Lee *et al.* 1990).

After the second mating season (third examination, 19 months post translocation), all adult koalas, except one female in ESF (Elisabeth), showed the presence of *Chlamydia* antibodies. The *Chlamydia*-negative female was often found in the vicinity of a translocated male. This was the only female with a live progeny at the end of the study. The reasons for her *Chlamydia*-negative status are unknown.

At the end of the study, the results of ELISA and cell culture appeared to be very similar among the three forests, but no clinical signs were evident during the study. The animals still appeared healthy as determined by weight measurement and physical condition. As previously demonstrated (Blanshard 1994; Carey *et al.* 2010)), infertile females may show no overt signs of infection; however, the disease appeared to have negative consequences on reproductive success in the second mating season as no *Chlamydia*-positive females bred successfully. The only koala (Elisabeth) with a live pouch-young after the second mating season in ESF did not show any chlamydial antibodies at the end of the study. The presence of a dead young in Elly's pouch was possibly related to her *C. pecorum* infection detected by PCR. As no post-mortem or microbiological examinations were

Table 2. Comparison of positive ELISA and DIF results among the three forests for the translocated koalas and three back-young.

Forests	*Chlamydia* antibodies (ELISA)		DIF or cell culture	
	2nd examination	Last examination	2nd examination	Last examination
Creswick	6/10 (60%)	5/6 (83%)	0/10 (0%)	4/6 (67%)
Enfield	2/10 (20%)	5/6 (83%)	0/10 (0%)	5/6 (83%)
Lal Lal	4/5 (80%)	5/5 (100%)	0/5 (0%)	2/4 (50%)

Table 3. Overall infection and health status of the translocated koalas. N=number of animals tested; F=females; M=males

Chlamydia tests

Time since release (months)	*Chlamydia* antibodies (ELISA) positive	DIF Positive	Cell culture positive	*Chlamydia* species (PCR)	Health status	Females N	Progeny N
0	0/30	N/A	N/A	N/A	Apparently good	20	0
6 April 1998	14/25 (56.0%) F= 9/16 M=3/9	0/25	N/A	N/A	Apparently good	16	6 live
19 May 1999	16/17 (94.1%) M=2	6/16 (37.5%)	5/16 (31.25%)	7/9 *C. pec.* only 1/9 *C. pec* and *C. pn.* 1/9 *C. pn.* only	Apparently good	12	1 live 1 dead in pouch

carried out, the cause of death of the pouch young was not established. Abortion or death of neonate was also noted in a previous translocation study on Phillip Island (Lee *et al.* 1990), and in pigs with a *C. pecorum* infection (Blanshard 1994). In both cases, no external signs of the disease were present.

The presence of *C. pecorum* in 8/9 and *C. pneumoniae* in 2/9 koalas tested, confirms what was indicated by other authors (Jackson *et al.* 1999; Koala Research Network (KRN) 2011; Kollipara *et al.* 2013; Patterson *et al.* 2015), that the former is more prevalent than the latter. It is not known, however, what the percentage occurrence of the two *Chlamydia* species was among the resident koala populations.

Progeny of affected koalas showed a high chlamydial antibody titre and the presence of chlamydial organism in the uro-genital tract. The detection of *C. pecorum* in the back-young of an infected mother is an indication that *C. pecorum* can be transmitted from mother to juvenile as previously suggested for koala populations in Queensland (Jackson et al. 1999). Previous studies found the presence of chlamydial organism in the rectum of female koalas (Brown 1987 in Brown and Woolcock 1990) and past authors (Brown and Woolcock 1990; Fleay 1937; Minchin 1937; Pournelle 1961) linked coprophagy to *Chlamydia* in young koalas. Jackson et al. (1999) detected high level of *C. pecorum* infection in both ocular and uro-genital sites in sexually immature koalas. Their study suggested that infection caused by *C. pecorum* could be transmitted from mother to newborn during birth or during pouch life.

Ethics of Translocation

This study has shown a high incidence of chlamydial infection amongst koalas translocated from a *Chlamydia*-free area to *Chlamydia*-positive sites. It is not clear whether, after the completion of this study, any koalas showed overt signs of the disease, or if some resistance to the bacterium developed. Longer-term studies are needed to establish the progression of *Chlamydia* in resident and translocated populations.

This management option raises ethical questions which need addressing (Whisson *et al.* 2012). As in many other studies (Dickens *et al.* 2009; Fraser *et al.* 2009; IUCN/SSC 2013), translocated animals in this research were infected by resident disease-positive animals. As translocation of koalas is still used in most Australian States for the management of koala populations, this outcome is of great concern from an animal welfare perspective at both individual and population levels. In the case of koalas in S-E Queensland, individuals whose chlamydial status is undetermined, are released to sites where the disease status is not fully ascertained. The Queensland Nature Conservation (Koala) Conservation Plan 2006 (Department of Environment and Heritage Protection 2014b) states that:

> *'Translocation of koalas will be considered only for scientific purposes, such as securing the viability of a population. Considerable scientific evidence is required to demonstrate the need for translocation. Translocation will not be considered for non-scientific endeavours, such as the removal of animals from land undergoing development'.*

However, translocations of koalas, are not undertaken for scientific purpose and/or to 'securing the viability of a population', but rather as a means of removing koalas from areas where development has been planned (Council of the City of Gold Coast 2015). As the recent modelling for S-E Queensland (Rhodes et al. 2015) has shown, one of the causes for the severe koala population decline is the impact of *Chlamydia* and other diseases.

Considering the finding of this and other research, translocating koalas of unknown *Chlamydia* status to sites where the disease status is also unknown, should be regarded as detrimental to the animals' health and welfare

and should only be the exception not the rule. It should only be undertaken with the greatest possible care and extensive monitoring and needs to be consistent with the Australasian Wildlife Management Society (AWMS) (2016) and IUCN/SSC (2013) guidelines.

Where to from here?

Even though this study was conducted in Victoria, its findings are strongly relevant to translocation of koalas as a management practice in other States. Considering the threatened status of koalas in Queensland, Australian Capital Territory and New South Wales, it is a priority to protect koalas' habitat rather than translocating to other sites to allow large scale development.

Translocated animals, in this study, were seropositive to *Chlamydia* after only six months, but did not display any overt physical signs, though, most females did not reproduce the second year after release. Not all *Chlamydia* infected koalas display clinical signs, and some populations are overabundant and overbrowsing their preferred food tree species (Menkhorst *et al.* 1998) despite the presence of the bacterium. There is a real concern that this bacterium is spread through populations before affected animals become infertile.

Research on the causes of *Chlamydia* is increasingly highlighting the relevance of stress on the infection rate and display of symptoms of various populations (Patterson *et al.* 2015), and other studies have linked diseases to the stress of translocation (Dickens *et al.* 2009; Fraser *et al.* 2009).

Aside from some observations, no detailed study has been carried out to determine if translocation and anthropogenic changes to koala habitat can cause stress, immune depression and the consequent overt expression of diseases (Brearley *et al.* 2013) such as *Chlamydia.*

Animal ethics

This research was approved by the Animal Ethics and Experimentation Committee, University of Ballarat (Permit No.97/004) as well as former DNRE Wildlife and National Parks Act 1975 (Permit No. 10000291).

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